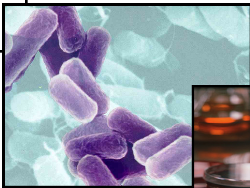

IBASM NEWSLETTER

Volume 16, Issue 1
August, 2013

Greetings from the President: Becky Sparks-Thissen

I hope you've had a productive and relaxing summer! I enjoyed meeting many of you at our meeting in April and look forward to seeing you again for next year's meeting. Congratulations to our poster award winners and to all who presented a talk and/or a poster! It was great to see all the exciting science you are involved in doing.

It is my pleasure to introduce **Nancy Magill** from Indiana University as our new President-Elect. I look forward to working with Nancy to plan next year's meeting.

Keep our annual meeting in mind as you plan out your schedules for the upcoming academic year. It will be held at Turkey Run State Park, March 28-29, 2014. As most of you already know, the annual meeting is a good place for undergraduate students, graduate students and postdocs to present their research. It can be a particularly good place for students from smaller institutions to meet students with similar interests or for students planning on giving a talk or poster at the general meeting in May to practice. We are in the process of planning for the meeting. Look for details in the next newsletter.

We are also looking for a new student representative at the graduate and undergraduate level. Please contact me for details on how to apply and what this position entails. (rlsparksth@usi.edu). Congratulations to **Breanna Brenneman,** who will be graduating from Ball State in December.

We will be announcing the next student grant competition soon. These grants support graduate student or undergraduate research and provide funds up to $1000. Be sure to keep your eyes open for this opportunity!

Finally, IBASM has a Facebook page (http://www.facebook.com/#!/IBASM). We will be posting information about grant opportunities and meeting information on this page. If you haven't already looked at our page, be sure to check it out.

Best wishes for the upcoming fall semester.

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Special Thanks to All Judges!

On behalf of all of the students in the poster competition I would like to express sincere appreciation to all of the members who volunteered their time to judge at the meeting.

Students were evaluated in 5 different categories: professional appearance, scientific thought, creativity, thoroughness and presentation (abstract, oral and poster). This was no easy task! Next time you see any of these persons please thank them for sweating through a very difficult challenge:

Team #1: Undergraduate division = *Dominique Galli (IUSD) and Christian Chauret (IU Kokomo)*

Team #2: MS division = *Richard Gregory (IUSD) and Tom Schwan (NIAID, NIH)*

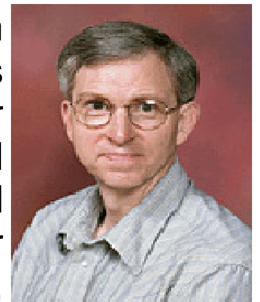
Team #3: Ph.D. division = *Doug Stemke (UI) and Tanya Soule (IPFW)*

From the Desk of Jim Mitchell-…Educational Representative

Students contributed a total of 20 posters and 7 oral presentations at the IBASM meeting. The quality of all the presentations was most excellent and it was very informative for me to see the range of different research areas. Cameron Unverferth (IU Southeast) won first place and Chris Coplen (IU Bloomington) second place in the undergraduate division.

Meiping Ye (IU School of Medicine) received the *Leland S. McClung* award for 1st place in MS division and Alexandra Okihiro (IUPU Fort Wayne) received 2nd place. David Welkie (PU) received the *Leland S. McClung* award for 1st place in PhD division and Shubham Chakravarty (IUPUI) received 2nd place. **Congratulations !!!**

The socializing which occurred during the open poster session was almost deafening at times, but a great opportunity for students to visit with each other and also interact with professionals who can provide valuable ideas and advice for future education and employment. All of us who viewed the poster segment and attended oral presentations look forward to even a greater number of participants next year, and I hope to possibly see students compete in the high school division. Winners received a certificate and will receive a monetary gift when a short paper is published in the IBASM newsletter.



2013 IBASM Award Recipient

Dr. Heather Bruns from Ball State University is this year's recipient of the IBASM Academic Teaching Award. Dr. Bruns is an Associate Professor in the department of biology who teaches undergraduate and graduate courses in the areas of cellular and molecular biology, virology, immunology and cell culture. A group of eleven current and former Ball State students had nominated Dr. Bruns for the award. The nomination letter described recent efforts by Dr. Bruns to switch to interactive teaching techniques that put an emphasis on student active learning. Dr. Bruns was also lauded as a role model to her female students demonstrating that women scientists can balance career and family life. Due to a scheduling conflict this spring Dr. Bruns will give her award presentation at the 2014 spring meeting at Turkey Run State Park.

Call for Nominations: IBASM Academic Research Award 2014

We call on all IBASM members to nominate their colleagues for the IBASM Academic Research Award 2014. At the time of nomination candidates should have been actively engaged in a nationally recognized research program for at least 5 years as a faculty member of an Indiana college or university. Evidence of a track record of scholarly work and significant external funding as a Principal Investigator will need to be provided. Please attach the nominee's short CV to the nomination letter. Note, the nominee does *not* have to be a member of the IBASM.

The IBASM Awards Committee consisting of Dominique M Galli (IU School of Dentistry), John McKillip (Ball State University), and Doug Stemke (University of Indianapolis) will select the awardee based on the nomination letter and information provided in the CV. The award will be presented at the IBASM Annual Spring Meeting in 2014 where the awardee will be expected to give an oral presentation. Please send your nominations to dgalli@iu.edu on or before November 1, 2013.

Call for Nominations: IBASM Academic Teaching Award 2014

Do you have a great professor who deserves a teaching award? We call on all student IBASM members to nominate their favorite lecturer/instructor for the IBASM Academic Teaching Award 2014. Your nomination letter should explain why you think your teacher deserves the award. Please provide as many details as possible. Also, your letter will carry more weight if you can get some of your peers to co-sign it.

The IBASM Awards Committee consisting of Dominique M Galli (IU School of Dentistry), John McKillip (Ball State University), and Doug Stemke (University of Indianapolis) will select the awardee based on your letter and additional information obtained from the nominee's departmental chair. The award will be presented at the IBASM Annual Spring Meeting in 2014 where the awardee will be expected to give an oral presentation. Note that the awardee must be a member of the IBASM at the time the award is received. Please send your nominations to dgalli@iupui.edu on or before November 1, 2013.

ASM Current Challenges

At the recent ASM Council Meeting at Denver, CO, outgoing president Jeff Miller described a series of challenges the society is facing. A draft of strategic issues and goals was presented to the council, which included the following:

- Revision of the ASM mission statement.
- Assessment of the current governance and program structure.
- Need for an image overhaul.
- Need to provide microbiology education to the public at large.
- Need for a strategy to recruit, retain and engage ASM members.
- Assessment of financial model.
- Formation of strategic partnerships with other societies at the national and international level.

Once these talking points are better defined, IBASM will seek input from its members to convey them to ASM.

2013 IBASM Membership Application/Renewal

If you have not done it already, it is time to pay your IBASM dues for 2013. You can do it either online when you pay your dues to the ASM National Organization (www.asm.org) or by using this form. Dues are $15.00 for non-students and $5.00 for students (per year). Please return the completed form with check, payable to IBASM, to

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Please check:
_____ New Member Application
_____ Renewal for 2013

and
_____ Student Member for 2013 ($ 5)
_____ Full Member for 2013 ($15)

Name:

Current Position & Title:

Institution:

Mailing Address (new address Yes / No ?) :

Phone:
Email:
Fax:
National ASM Member #:

Background

Highest Degree:
Institution:
Professional Interests:

McClung First Place Graduate (M.S. Division) Winner

The Second Messenger cyclic-di-AMP Controls Cell Growth and Virulence Production of *Borrelia burgdorferi*

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Introduction

Microbes have diverse signaling strategies to response quickly to the environmental changes. Cyclic-di-adenosine monophosphate (*c*-di-AMP) is a most recently discovered second messenger, first discovered in *Bacillus subtilis* in 2008 (8). *C*-di-AMP is synthesized from two molecules of ATP by *c*-di-AMP synthase with a conserved DAC (diadenylate cyclase) domain, and is hydrolyzed into 5'-pApA by phosphodiesterase (PDE) with a conserved DHH/DHHA1 domain. The DAC are often linked with domains that have diverse functions, indicating its important roles in diverse cellular processes (2, 7). Reports have shown that *c*-di-AMP is essential for bacterial cell wall synthesis, beta-lactam antibiotic resistance as well as pathogenesis in certain bacteria (3-6). However, the mechanism of *c*-di-AMP signaling is by large unknown, especially in Gram negative bacteria.

Experimental Methods

Both in vivo and in vitro experiments were conducted to illustrate the functions of BB0619 in *Borrelia burgdorferi*. For in vitro evidence, the bb0619 gene was over expressed in *E.coli* and purified to homogeneity using a Ni-NTA column. The purified proteins were further used for the enzyme assay of c-di-AMP phosphodiesterase and characterization. To further determine the role of c-di-AMP in the enzootic cycle of *B. burgdorferi* in vivo, a bb0619-deficient *B. burgdorferi* strain was generated by replacing the wild type chromosomal bb0619 with a disrupted gene via homologous recombination (Fig 2.). The phenotypes and virulence of the mutant strain was subsequently investigated using either mice or dialysis membrane chamber (DMC) model.

Results & Discussion

I. *bb0619* is the single *c*-di-AMP PDE gene in the *B. burgdorferi* genome. The genome of the Lyme disease pathogen *B.burgdorferi*, encodes a single diadenylate cyclase gene (*bb0008*) and a single phosphodiesterase gene (*bb0619*), predicted responsible for *c*-di-AMP synthesis and degradation in *B. burgdorferi*, respectively. Bioinformatics analysis of the genome of *B. burgdorferi* B31 indicated that bb0619 has a typical DHH domain, which was conserved among all phosphoesterases.

II. BB0619 is the specific *c*-di-AMP phosphodiesterase of *B. burgdorferi*. BB0619-His tagged protein was overexpressed and purified. The recombinant BB0619 demonstrated specifically activities for c-di-AMP degradation. The product of the enzyme catalyzed was confirmed to be the predicted 5'-pApA by HPLC analysis (Fig 1.). Enzyme characterization indicated the phosphodiesterase activity was metal dependent. It preferred divalent metal ions such as Mn^{2+} and Mg^{2+} and alkaline conditions with the optimal pH between 8.0 to 10.0. The biochemical character of BB0619 was similar with its homologue YybT in *Bacillus*, indicating its same origination.

III. The *bb0619* gene was successfully inactivated in the presence of an extra copy of IPTG inducible *bb0619*. Gene *bb0619* is essential for Borrelia since we cannot directly inactive it. Alternatively, we construct a shuttle vector with pQE30 promoted *bb0619*, which can be induced by IPTG. The plasmid, PQE30-bb0619 was transformed into *Borrelia* to get a strain carrying an extra copy of *bb0619*. The resulting strain *Borrelia* [PQE30-bb0619] was subsequently used for *bb0619* gene inactivation, with a suicide plasmid pMP004. The strategy of gene inactivation was shown in Fig 2 A and the *bb0619* mutant was confirmed by PCR screening from the *Borrelia* transformants (Fig 2B).

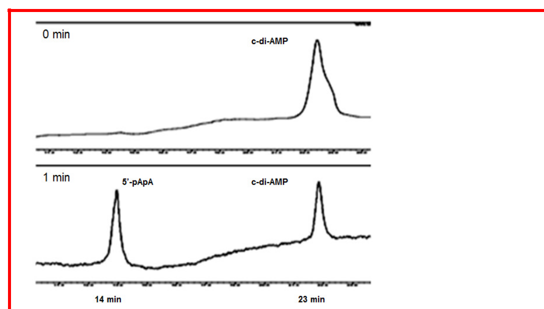

Fig 1. Characterization of the c-di-AMP hydrolytic activity of the *B. burgdorferi* BB0619 *in vitro*. c-di-AMP hydrolysis by BB0619 (YybT) monitored by HPLC. The reaction mixture contained 0.5 μM YybT and 50 μM c-di-AMP in the buffer: 20 mM Tris, pH 8.0, 80 mM KCl, 10 mM metal cation (2.5 mM for Mn^{2+}). Temperature 37°C. Nucleotides were separated and analyzed using the reversed phase HPLC (Summit HPLC System, Dionex, Sunnyvale, CA), 15 cm × 4.6 mm Supelcosil LC-18-T column (Sigma).

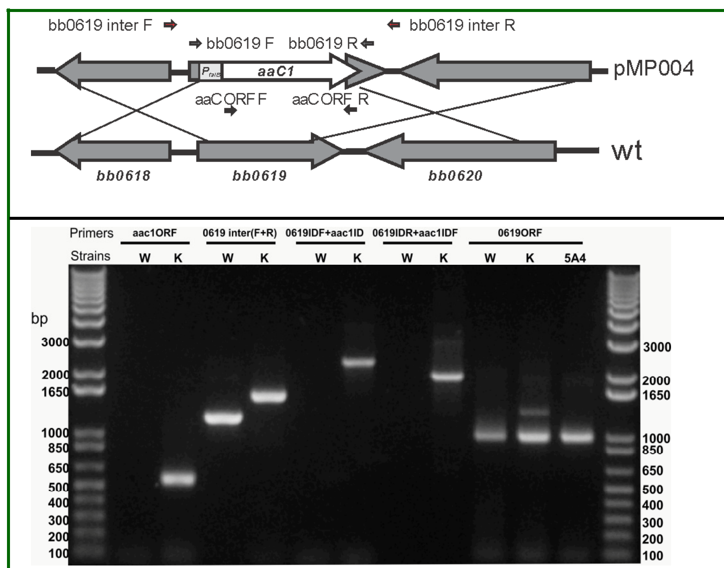

Fig 2. Construction of the bb0619 mutant. A) strategy for constuction of the mutant. Arrows indicate the approximate positons of the primers used for PCR analysis. B) PCR analysis of the strains. The specific primer pairs used in PCR are indicated above lanes. W represents wild type and K indicate gene knockout strain.

IV. Inactivation of *bb0619* significantly impaired the growth of *B. burgdorferi*. The growth phenotype of *bb0619* mutant was examined in vitro. And *bb0619* mutant was impaired in growth in the Barbour-Stoenner-Kelly-II (BSK-II) medium and cannot reach the same cell density as the parent wild type strain. Furthermore, it also exhibited a defect in cell division, and a longer cell shape was observed in the microscope. We hypothesis that c-di-AMP was involved in the regulation of *Borrelia* cell wall synthesis, and the accumulation of c-di-AMP in the cell affect bacterial growth rate and cell wall stability, which was consistent with reports from *S. aureus* (1)and *B. subtilis*(4).

V. The *bb0619* mutant had defect in producing the major virulence factor OspC, due to an impaired expression of BosR, a transcriptional activator. The *bb0619* mutant was unable to express the major virulence factor OspC, evidenced by SDS-PAGE and immunoblotting analysis (Fig 3). Moreover, the down regulation of OspC was caused by the reduction of BosR and RpoS (Fig 3B). This indicated that high level of c-di-GMP reduced the BosR level, which, in turn attenuated the expression of OspC via RpoS.

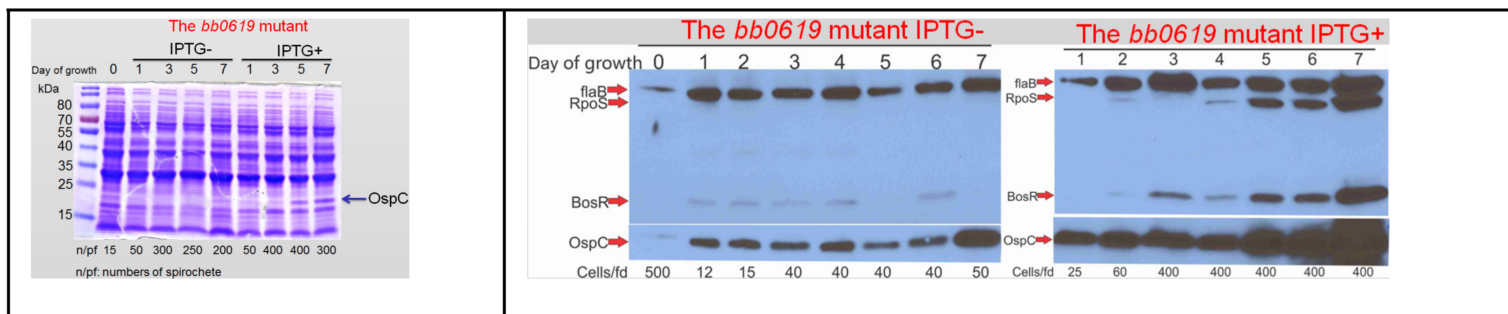

Fig 3. Inactivation of bb0619 downregulates RpoS and OspC levels via BosR. A) SDS-PAGE of whole cell lysates of related strains' late-log culure. B) Immunolblot with monoclonal antibodies. The initial cell density was 3×10^5 cells/ml for each strain. Spirochetes were enumerated under dark-field microscopy and the cell densities were shown under the lanes (spirochetes per field, pf). The treatment without IPTG (IPTG-) indicates *bb0619* mutant cultures, while IPTG+ indicates wild type cultures.

VI. *bb0619* was required for *Borrelia* growth *in vivo*. To determine the role of c-di-AMP and Bb0619 in mammalian infection, we needle-inoculated groups of mice with various *B.burgdorferi* strains. Two weeks post inoculation, ear punch biopsies were cultured in BSKII medium. Both the wild type and *bb0619* mutant with IPTG (complement strain) show positive results indicating the presence of spirochetes, while the *bb0619* mutant strain without IPTG cannot infect

mice. We also utilized DMC cultivation system for the investigation of the mutant's infection ability. The results also shown the mutant strain without IPTG cannot establish the infection process in DMC model. These data indicates that Bb0619 is essential for the mammalian survival and higher concentration of c-di-AMP impaired the virulence of the pathogen.

VII. Model for the c-di-AMP signaling system in *B. burgdorferi.* Based on our data shown above, it can be concluded that BB0619 is the specific c-di-AMP phosphodiesterase of *B. burgdorferi*, and *bb0619*-deficient *B. burgdorferi* was impaired in growth and cell division. The bb0619 mutant was unable to express the major virulence factor OspC, due to a reduced BosR level, while the in vivo conditions could not rescue the growth of the *bb0619* mutant. A model for the c-di-AMP signaling system was predicted as shown in Fig 4. To the best of our knowledge, our study is the first time to explore the function of c-di-AMP in Gram negative bacteria, and the findings will undoubtedly improve our understanding of the c-di-AMP signaling in diverse bacteria.

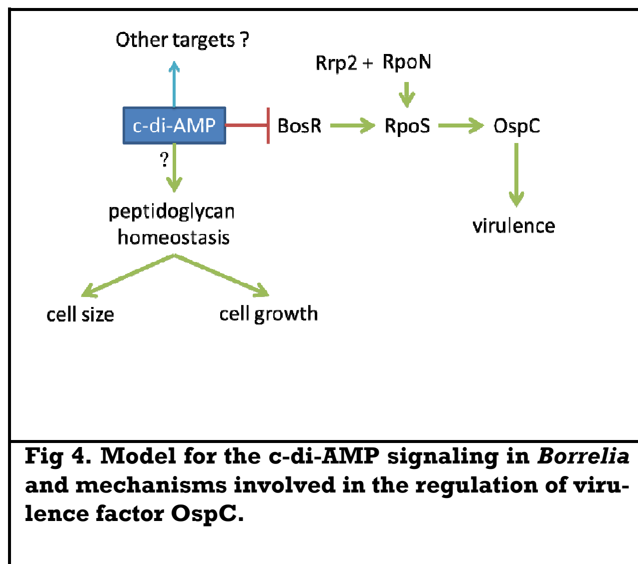

Fig 4. Model for the c-di-AMP signaling in *Borrelia* and mechanisms involved in the regulation of virulence factor OspC.

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Meiping Ye receiving her award from IBASM's President **Dr. Rebecca Sparks-Thissen**

Second Place (Ph.D. Division) Winner

The Regulatory Protein AlgR Influences *Pseudomonas aeruginosa* Pathogenesis on Airway Cells

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Introduction and Significance

Pseudomonas aeruginosa chronically colonizes the lungs of individuals with cystic fibrosis (CF) (2). Evidence suggests that this infection process involves the transition of *P. aeruginosa* from an acute, virulent phenotype to a more chronic lifestyle typified by the formation of biofilms within the airways (1, 2, 3). Our studies indicate that this reduction in virulence observed in biofilm forming lifestyle of *P. aeruginosa* is influenced by the genes *algR*, involved in alginate synthesis (4) and *mgtE*, an Mg transporter that modulates the Type 3 Secretion System (T3SS) (2). We have further observed that AlgR might have a negative regulatory effect on *mgtE* transcription. Using a previously developed novel tissue culture model system for the development of *P. aeruginosa* biofilms directly on cultured human CF-derived Broncho Epithelial (CFBE) cells (1, 2) we have also found that AlgR has significant roles in mediating adherence of *P. aeruginosa* on human airway cells and subsequent biofilm formation.

Materials & Methods

All mutants of the *Pseudomonas aeruginosa* (PA14) wild type strain and complementation plasmids were made by an allelic exchange technique using Yeast genetics (2). Biofilm on plastic was assayed by crystal violet stain as described previously (5). Biofilm and cytotoxicity assays on CFBE cells were done as previously described (1, 2). Beta galactosidase (Miller) assay was also performed as described in the literature (2).

Results & Discussion

The gene *algR* is involved with alginate synthesis in *P. aeruginosa* (4). Since the chief components of the biofilm formed by *P. aeruginosa* are complex polysaccharides like alginate, it can be hypothesized that AlgR may have an important role in biofilm formation. We found that biofilm formation on plastic is somewhat impaired in the PA14 strain which had the *algR* gene knocked out (Fig. 1). Subsequently, we infected human airway cells with wild-type (WT) *P. aeruginosa* strain PA14 and PA14 with a*lgR* deleted and found remarkably less adherence and biofilm formation by the latter. When the AlgR deletion mutant was complemented using a plasmid (pMQ72) that had the *algR* gene cloned into it, adherence and biofilm formation was significantly restored again (Fig. 2).

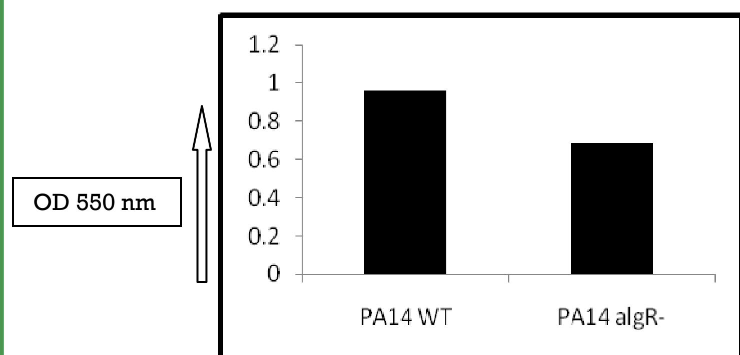

Figure 1. Biofilm formation on plastic by wild-type (WT) and Δ algR Pseudomonas aeruginosa (PA14) strains

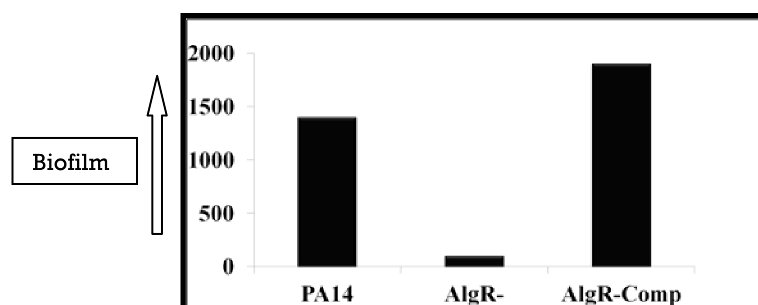

Figure 2. Biofilm formation on CFBE cells by wild-type, Δ*algR* and Δ*algR Pseudomonas aeruginosa* (PA14) complemented with *algR* complementation plasmid.

It has been reported that MgtE might down regulate T3SS expression in *P. aeruginosa*, during its course of infection in the CF lung environment (2). We have observed that AlgR, in addition to being a virulence modulator in CF related *P. aeruginosa* infection, disrupts the cytotoxicity phenotype of MgtE, suggesting a possible complex interaction between AlgR, MgtE and the T3SS (Fig. 3). While trying to further elucidate the relationship between AlgR and MgtE, it has been intriguingly observed, that AlgR might be negatively regulating *mgtE* transcription but the reverse is not evident (Fig. 4).

Taken together, this study provides useful insight into the molecular mechanisms that enable *P. aeruginosa* to establish and maintain biofilms in the lung environment of CF patients. Elucidation such molecular networks would provide important therapeutic cues for preventing and treating CF related *P. aeruginosa* lung infection.

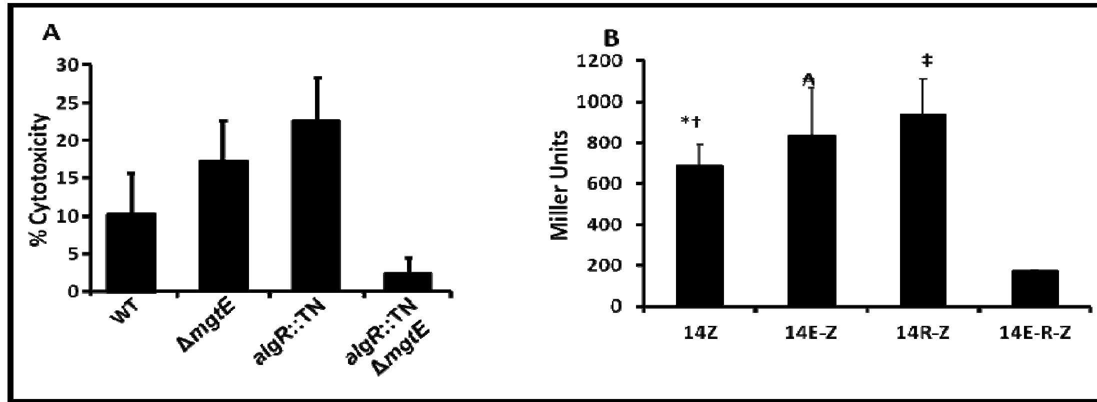

Figure 3. AlgR disrupts the virulence modulatory phenotype of *mgtE*. **(A)** The Δ*mgtE* and *algR*::Tn strains both display increased cytotoxicity, compared to wild type (WT), when grown as biofilms on cultured CF bronchial epithelial cells. However, mutation of both in the same strain leads to decreased cytotoxicity. Data were assayed in triplicate. **(B)** For examining the T3SS promoter activity in the PA14 strain under different mutant conditions, Beta galactosidase activity (Miller units) was measured from the $P_{exsD\text{-}lacZ}$ construct in the wild-type PA14 (14Z), Δ*mgtE* (14E-Z), Δ*algR* (14R-Z) and Δ*mgtE* Δ*algR* (14E-R-Z) strains grown as planktonic, broth cultures. The data are representative of five independent experiments. P<0.05, compared to 14R-Z strain; †, P<0.05, compared to 14E-R-Z strain; □, P<0.05, compared to 14E-R-Z strain; ‡, P<0.05, compared to 14E-R-Z strain.

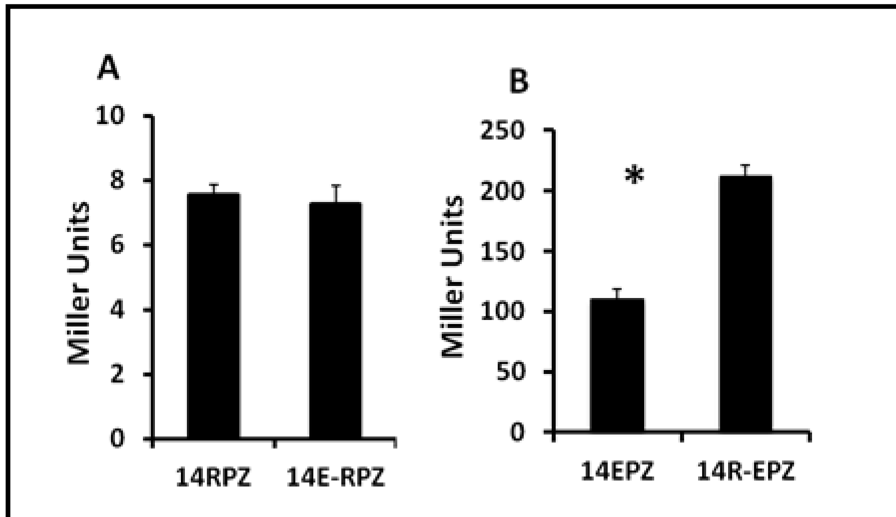

Figure 4. AlgR negatively regulates *mgtE* promoter activity, but no significant effect of MgtE on *algR* promoter activity. **(A)** Beta galactosidase activity (Miller units) was measured from the $P_{algR\text{-}lacZ}$ construct in the wild-type PA14 (RPZ) and Δ*mgtE* (14E-RPZ) strains grown as planktonic, broth cultures. Data are representative of three independent experiments, each containing triplicate or quadruplicate samples. **(B)** Transcriptional activity of the *mgtE* promoter was analysed by the Beta galactosidase test (Miller units) using the $P_{mgtE\text{-}lacZ}$ construct in the wild-type PA14 (EPZ) and Δ*algR* (14R-EPZ) strains grown as planktonic, broth cultures. Data are representative of three independent experiments, each containing triplicate or quadruplicate samples.

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First Place Undergraduate Winner

Identification and Characterization of Black Pigment in Environmental Bacteria

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New Albany, IN

Introduction

Environmental soil and sand samples obtained during a field geology expedition to the Hashemite Kingdom of Jordan were analyzed to determine desert microbial communities. Investigation of the microbial community revealed the presence of a novel black pigment forming bacterium. Preliminary work on this bacterium identified it as being of the *Planococcus* genus, found in a wide variety of environmental conditions and often associated with marine environments. Black pigment formation by this bacterium isolate is significant, in part, because black pigmentation is unknown in members of the *Planococcus* genus and uncommon in bacteria as a whole. This study will seek to characterize the organism via standard biochemical assays, analyzing the 16S ribosomal DNA sequence, analyzing fatty acid methyl ester (FAME) content, and studying the phenotypic growth patterns of the bacterium.

Methods

Environmental cultures were grown on 5% NaCl marine agar plates, then isolated based on morphology and incubated at 25°C. The 16D rDNA sequence was obtained using 27f (5-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-ACCTTGTTACGACTT-3') universal primers[1] and analyzed using Chromas (Technelysium, South Brisbane, Australia). A BLAST search was conducted to find related organisms[2]. A fatty acid methyl ester analysis (MIDI, Newark, DE) was conducted to further characterize the organism. Sodium chloride tolerance tests were conducted with %NaCl ranging from 2%-20%. Diagnostic assays, including gram staining, endospore staining, citrate, lysine decarboxylase, oxidase, catalase, gelatin hydrolysis, casein hydrolysis, lactose fermentation, temperature tolerance, motility and anaerobic growth tests[3] were conducted on the organism for additional characterization data.

Initial attempts to extract and analyze the black pigment formed by the organism were conducted using organic solvents, including acetic acid, acetone, deionized water, dicholormethane, and ethanol. Additionally, 0.1% Tween 20 solution and 1% Triton X 100 solution were employed to attempt to extract the pigment from the membrane. A solution of 0.4M EDTA was used to precipitate any metals that might be associated with the formation of the black pigment. Each of these experimental conditions were repeated in acidic conditions using 0.1M HCl and in basic conditions using 0.1M NaOH.

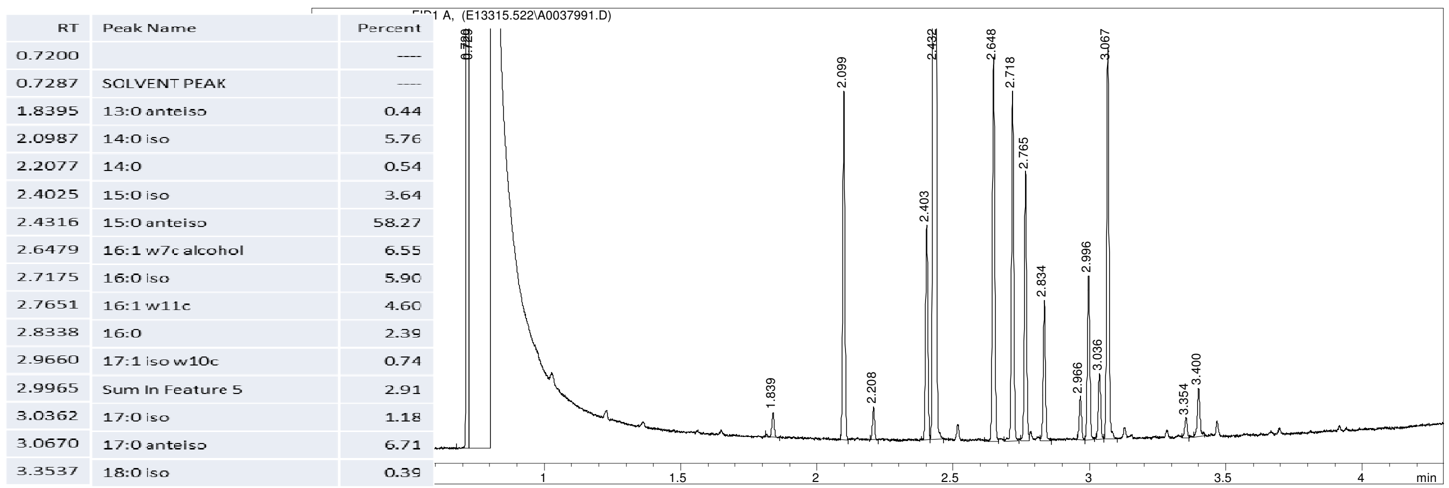
The source sand was analyzed to determine mineral content as a means of assessing the organism's habitat and potential nutrient sources. Grain size and maturity of the sand was evaluated under cross-polar microscopy. Mineral content was determined by inductively coupled argon plasma spectrophotometry (ICAP) using the Mehlich III extraction method[4].

Results

16S data analyzed with Chromas showed 62% identity with the *Planococcus* genus at a 93% confidence when submitted to BLAST search. FAME analysis supported this finding, indicating a 98% similarity to *Planococcus citreus*. There was not a significant difference in cell membrane composition from light phase to dark phase seen in the FAME analysis (Figure 1). The black pigment may be a response from exposure to sodium chloride (data not shown), and is supported by source sand analysis data which revealed an environment of 3.8% sodium chloride content. Aerobic incubation in the dark after one week on marine agar initially produced white colonies, then black pigmentation developed, progressing from light brown to an alternating light/dark brown ring morphology and finally to black after two weeks of growth (Figure 2). Additionally, extraction attempts using EDTA indicate the pigment is potentially not a metal.

Conclusion

16S and FAME analysis support the conclusion that this organism is a previously undescribed species belonging to the *Planococcus* genus. Literature review also supports the black pigment production is not previously described within the *Planococcus* genus[5-11]. Data from the diagnostic characterization tests shows the organism has comparable metabolic



RT	Peak Name	Percent
0.7200		----
0.7287	SOLVENT PEAK	----
1.8395	13:0 anteiso	0.44
2.0987	14:0 iso	5.76
2.2077	14:0	0.54
2.4025	15:0 iso	3.64
2.4316	15:0 anteiso	58.27
2.6479	16:1 w7c alcohol	6.55
2.7175	16:0 iso	5.90
2.7651	16:1 w11c	4.60
2.8338	16:0	2.39
2.9660	17:1 iso w10c	0.74
2.9965	Sum In Feature 5	2.91
3.0362	17:0 iso	1.18
3.0670	17:0 anteiso	6.71
3.3537	18:0 iso	0.39

Figure 1: Fatty Acid Methyl Ester (FAME) analysis of the cell membrane composition of the novel bacterium.

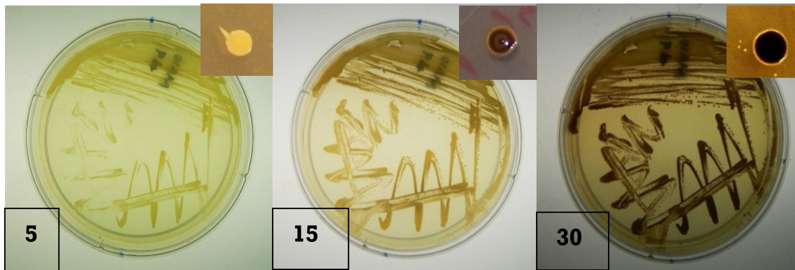


Figure 2: Morphology tracking of the unknown bacterium showing color change over time. The number in the lower left corner indicates days after inoculation. The insert in the upper right gives a detailed view of the developmental stages of the pigment as it progresses from white to a brown ringed structure to black.

activity to other described members of the *Planococcus* genus[5-9]. The bacterium appears to have the ability to ferment lactose, is catalase and gram positive, motile, and does not appear to form endospores (Table 1 and Figure 3). Attempts to extract the pigment from the cell using aqueous, chelating, and organic solvents have been unsuccessful.

A novel bacterium has been isolated and cultured from the desert region of Hashemite Kingdom of Jordan. Growth of this bacterium produced a black pigmentation. 16S ribosomal DNA sequencing and FAME analysis revealed that this organism is a previously undescribed species belonging to the *Planococcus* genus. Literature review also supports the black pigment production is not previously described within the *Planococcus* genus[5-11]. Biochemical analysis demonstrated this bacterium to gram positive to gram variable, catalase positive, and motile positive. Further experiments continue in the investigation of the black pigment and its role in this novel organism

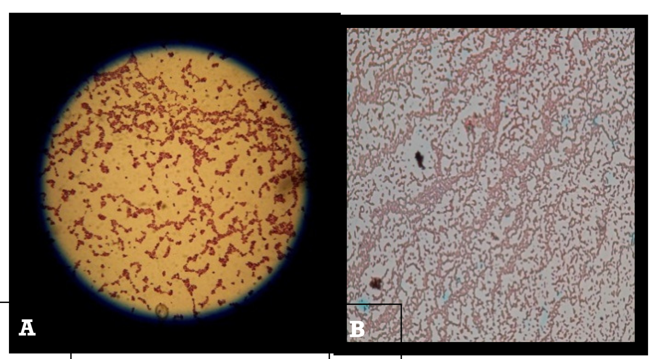


Figure 3: Gram staining results indicating gram$^+$ to gram variable cocci. B: Endospore staining using malachite green, indicating no endospores present.

Test	Result
Gram Stain	Positive to Variable
Endospore Stain	Negative
Citrate	Negative
Lysine Decarboxy-lase	Negative
Oxidase	Negative
Catalase	Positive
Gelatin Hydrolysis	Negative
Casein Hydrolysis	Negative
Lactose Fermenta-tion	Positive
Motility	Positive
Temperature Range	25°C-55°C
NaCl Tolerance	0%-15%

Table 1: Results of diagnostic assays conducted on unknown bacterium.

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Cameron Unverferth receiving his award from IBASM's President **Dr. Rebecca Sparks-Thissen**

Highlights from the Journals of the ASM (from ASM Tipsheet)

Microbial Changes Regulate Function of Entire Ecosystems

A major question in ecology has centered on the role of microbes in regulating ecosystem function. Now, in research published ahead of print in the journal *Applied and Environmental Microbiology*, Brajesh Singh of the University of Western Sydney, Australia, and collaborators show how changes in the populations of methanotrophic bacteria can have consequences for methane mitigation at ecosystem levels.

"Ecological theories developed for macro-ecology can explain the microbial regulation of the methane cycle," says Singh.

In the study, as grasslands, bogs, and moors became forested, a group of type II methanotrophic bacterium, known as USC alpha, became dominant on all three land use types, replacing other methanotrophic microbes, and oxidizing, thus mitigating methane, a powerful greenhouse gas, explains Singh. "The change happened because we changed the niches of the microbial community."

The pre-eminence of USC alpha bacteria in this process demonstrates that the so-called "selection hypothesis" from macro-ecology "explains the changes the investigators saw in the soil functions of their land-use types," says Singh. The selection hypothesis states that a small number of key species, rather than all species present determine key functions in ecosystems. "This knowledge could provide the basis for incorporation of microbial data into predictive models, as has been done for plant communities," he says.

"Evidence of microbial regulation of the biogeochemical cycle provides the basis for including microbial data in predictive models studying the effects of global changes," says Singh.

Singh warns that one should not take the results to mean that biodiversity is not important. Without microbial biodiversity, the raw materials—different microbial species with different capabilities—for adapting to changes in the environment would be unavailable, he says

(L. Nazaries, Y. Pan, L. Bodrossy, E.M. Baggs, P. Millard, J.C. Murrell, and B.K. Singh, 2013. Evidence of microbial regulation of biogeochemical cycles from a study on methane flux and land use change. Appl. Environ. Microbiol.)

High-Salt Diet and Ulcer Bug Combine to Increase Risk of Cancer

Numerous epidemiologic studies have shown that a diet high in salt is associated with an increased risk of gastric cancer. Now Timothy L. Cover and colleagues of Vanderbilt University show that high dietary salt combined with infection by the ulcer-causing bacterium *Helicobacter pylori* greatly increases the risk of cancer. The study was published ahead of print in the journal *Infection and Immunity.*

In the study, the researchers infected Mongolian gerbils with *H. pylori.* One set of gerbils received a regular diet; the other, a high salt diet. At the end of the experiment the researchers analyzed the animals' stomach tissues. Every animal on the high salt diet developed cancer, compared with just 58 percent of those on the regular diet.

It appears development of gastric cancer required the presence of a particular bacterial oncoprotein, known as CagA, which is produced by *H. pylori*. Gastric cancer did not develop in animals on the high salt diet that were infected with a mutant *H. pylori* which did not produce CagA. In earlier studies, Cover and others had shown that culturing *H. pylori* in a high salt environment boosts production of CagA. "This was one of the driving forces that led us to undertake the current studies," says Cover.

The investigators note that while no studies, to their knowledge, have examined relationships among a high salt diet, and infection with *H. pylori* expressing *cagA*, "in several parts of the world that have high rates of gastric cancer, there is a high prevalence of *cagA*+ strains and a large proportion of the population consumes a high-salt diet."

The investigators also detected significantly higher levels of gastric inflammation in *H. pylori*-infected gerbils on a high salt diet than in those on a regular diet, a finding which Cover says is relevant to many types of cancer. They also showed that transcription of various inflammatory cytokines, such as interleukin 1-beta, are elevated in the former as compared to the latter, suggesting that "these factors may contribute to the increased inflammation and increased gastric risk that accompany a high salt diet," says Cover.

(J.A. Gaddy, J.N. Radin, J.T. Loh, F. Zhang, M.K. Washington, R.M. Peek, Jr., H.M.S. Algood, and T.L. Cover, 2013. High dietary salt intake exacerbates *Helicobacter pylori*-induced gastric carcinogenesis. Infect. Immun.)

Microbiology in the News

(from: http://www.eurekalert.org/bysubject/index.php?kw=33)

From harmless colonizers to virulent pathogens: UB microbiologists identify what triggers disease

mBio

August 6, 2013

The bacteria *Streptococcus pneumoniae* harmlessly colonizes the mucous linings of throats and noses in most people, only becoming virulent when they leave those comfortable surroundings. Now, University of Buffalo researchers reveal how that happens.

http://www.buffalo.edu/news/releases/2013/08/002.html

New design may produce heartier, more effective salmonella-based vaccines

Journal of Bacteriology

August 6, 2013

Through genetic manipulation, the species *S. typhi* can be rendered harmless and used in vaccines in order to prevent, rather than cause illness. ASU scientists describe efforts to improve the effectiveness of a Recombinant Attenuated Salmonella Vaccine by modifying its ability to survive the hostile environment of the stomach.

http://www.biodesign.asu.edu/news/new-design-may-produce-heartier-more-effective-vaccine

Percentage of cancers linked to viruses potentially over-estimated

Journal of Virology

August 5, 2013

The results of a large-scale analysis of the association between DNA viruses and human malignancies suggest that many of the most common cancers are not associated with DNA viruses. The findings, published in the Aug. 2013 issue of the Journal of Virology, challenge earlier studies suggesting as high as 40 percent of tumors are caused by viruses.

http://bit.ly/asmtip0713d

Important Dates

February 2014:	Registration form due for Annual IBASM meeting
March 2014:	Abstract form due for Annual IBASM meeting
March 28-29, 2014:	Annual IBASM meeting at Turkey Run State Park
May 17-20, 2014:	114th Annual Meeting of the ASM, Boston, MA

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